Attorney's Docket No.: 56446-20016.20/-027003 /D1350-2

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Walter Callen et al. Art Unit : 1652
Serial No. : 09/656,309 Examiner : Richard Hutson, Ph.D.
Filed : September 6, 2000
Title : ENZYMES HAVING HIGH TEMPERATURE POLYMERASE ACTIVITY AND METHODS OF USE THEREOF

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. § 1.132

Sir:

1. I, Walter Callen, am a co-inventor with Eric J. Mathur, on the above-identified patent application.

2. I am an expert in the field of molecular biology and enzyme development and was an expert at the time of the invention. I am presently employed as a research scientist at Diversa Corporation, San Diego, CA, assignee of the above-referenced patent application. My resume is attached as documentation of my credentials.

3. I declare that assays for identifying nucleic acids that encode polypeptides having polymerase activity were conventional and routine in the art at the time of the invention. Procedures for identifying polypeptides having polymerase activity, including thermotolerant or thermostable polymerase activity under varying conditions, such as varying temperature and pH conditions, were conventional and routine in the art at the time of the invention. For example, by 1996, high through-put *in vivo* (e.g., whole cell) nucleic acid expression and polymerase activity screening protocols were well known in the art. In particular, high through-put methods to

screen for polymerase activity, such as polymerase chain reaction (PCR), were well known in the art. Making polypeptide-encoding nucleic acids of different sizes or varying sequences based on an exemplary sequence, and screening them for polymerase activity under various conditions was a predictable art at the time of the invention. Methods for determining sequence identity were also routine and well known in the art at the time of the invention. Accordingly, one of ordinary skill in the art using the teaching of the specification would have been able to ascertain what polymerase-encoding nucleic acids, including nucleic acids of varying sizes and sequences, were within the scope of the claims with reasonable clarity to recognize that Applicants' were in possession of the invention at the time of filing.

4. I declare that procedures for modifying nucleic acids were conventional and routine in the art at the time of the invention. One of ordinary skill in the art using the teaching of the specification would have been able to select any known method of modifying nucleic acids, including any one or a combination of exemplary methods set forth in the specification, including error-prone PCR, shuffling, oligonucleotide-directed mutagenesis, assembly PCR, sexual PCR mutagenesis, in vivo mutagenesis, cassette mutagenesis, recursive ensemble mutagenesis, exponential ensemble mutagenesis, site-specific mutagenesis, ligation reassembly or Gene Site Saturatation Mutagenesis™ (GSSM™), to make a variant of SEQ ID NO:1, or a variant of a nucleic acid having 70% sequence identity to SEQ ID NO:1, or a variant of a nucleic acid comprising a fragment of at least 30 consecutive nucleotides of a sequence having at least 70% identity to the sequence set forth in SEQ ID NO:1, to practice the methods of the invention without undue experimentation.

5. I declare that methods for changing, or varying, nucleic acids sequences were well known in the art at the time of the invention. It was considered routine by one skilled in the art at the time of the invention to screen for multiple substitutions or multiple modifications in a nucleic acid sequence for functional variations, e.g., variant nucleic acids that encode a polymerase enzyme. For example, high through-put methods for screening for polymerase activity, such as polymerase chain reaction (PCR), were well known in the art. While the numbers of samples needed to be screened may have been high, the screening procedures were

routine and successful results (e.g., finding variant nucleic acids encoding polymerases, such as thermostable polymerases) predictable. Accordingly, at the time of the invention it would have been considered routine by one skilled in the art to generate and screen multiple substitutions or multiple modifications in a nucleic acid sequence and predictably generate functional variants.

6. I declare that it was not necessary for the skilled artisan to understand which specific regions of polymerase structure may be modified without affecting function or activity, or, which specific regions of polymerase structure should be modified to generate altered enzyme activity, to practice the methods of the invention because methods for modifying sequences, generating polymerase-encoding sequences, and screening for polymerase activity at the time of the invention were routine and predictable. For example, methods for sequence modifications were sufficiently routine and predictable at the time of the invention to predictably generate polymerase-encoding sequences without need of knowing which specific regions of polymerase structure affect polymerase function or activity. For example, on pages 32 to 35, the specification gives a detailed description of such an exemplary method – a method for sequence modification called Gene Site Saturation Mutagenesis™ (GSSM™). In one aspect of GSSM™, degenerate oligonucleotides comprising degenerate N,N,N cassettes can be used for subjecting each original codon in a parental polynucleotide template to a full range of codon substitutions. Thus, GSSM™ allows for mutagenizing each and every amino acid position in a parental polypeptide to generate amino acid changes that can be routinely screening for their effect on activity. Methods known for modifying nucleic acid sequences such as GSSM™ known at the time of the invention made methods that require previous knowledge of protein tertiary structure, active sites and the like obsolete and unnecessary. Accordingly, using methods known in the art at the time of the invention, e.g., GSSM™, it would not have been necessary to understand which specific regions of polymerase structure can be modified to generate variant enzymes to practice the methods of the invention.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are

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punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Respectfully submitted

Date: 4/20/04

Walter Callen

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PROFESSIONAL EXPERIENCE

Fourteen years of experience in as a molecular biologist in an industrial setting.

Staff Scientist II - Diversa Corporation, San Diego CA. 6/94 - present.
Responsible for the development of screening methods and discovery of novel industrial enzyme clones to fulfill partnership agreements. The overexpression of target enzymes in bacterial hosts. Radiation Safety Officer. Head of Hybridization Department. Supervisor of two technicians.

Research Accomplishments:

- Discovery of over 500 enzyme clones
- Representing over 50% of Diversa's enzyme clones
- Among these are DNA polymerases, esterases, and glycosidases
- Assisted in the screening for dehalogenases, insecticides and bioactive pathway clones
- Utilizing high-throughput screening methods

Senior Research Associate - Stratagene Cloning Systems, La Jolla CA. 8/89 - 6/94.
Responsible for research and development of molecular biological products. Laboratory Manager. Chemical Hygiene Officer.

Research Accomplishments:

- Cloning of six DNA modifying enzymes
- The Clearcut Miniprep kit
- The Cyclist, cycle sequencing kit
- DNA primer synthesis via Hexamer Ligation
- Kb DNA size markers
- ExoMeth sequencing kit

Graduate Assistant - San Diego State University, San Diego, CA. 9/85 - 8/89.
Instructor for college cell biology laboratory course and grading lab reports. Prepared and presented course materials, preparing exams and assigning grades for beginning college biology laboratory course. Responsible for ordering supplies, washing glassware, autoclaving and P1 waste disposal for molecular biology laboratory.

EDUCATION

Master of Science, Molecular Biology. San Diego State University, San Diego, CA. July 1989. Masters Thesis - Molecular Characterization of M308 a Flightless Mutant of Drosophila melanogaster.

Bachelor of Arts, Chemistry. Bachelor of Arts, Biology with emphasis in **Molecular Biology.** Humboldt State University, Arcata, CA. June 1985.

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AU Patent No. 735082 Callen, Walter; Mathur, Eric. Isolation and Identification of Novel Polymerases.

US Patent No. 6,492,511 Callen, Walter; Mathur, Eric. Isolation and Identification of Novel Polymerases.

US Patent No. 5,948,666 Callen, Walter; Mathur, Eric. Isolation and Identification of Novel Polymerases.

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Sehgal AC, Callen W, Mathur EJ, Short JM, Kelly RM. Carboxylesterase from Sulfolobus solfataricus P1. Methods Enzymol 2001 330:461-71.

Cady SG, Bauer MW, Callen W, Snead MA, Mathur EJ, Short JM, Kelly RM. Beta-Endoglucanase from Pyrococcus furiosus. Methods Enzymol 2001 330:346-54

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Michael W. Bauer, Lance E. Driskill, Walter Callen, Marjory A. Snead. Eric J. Mathur, and Robert M. Kelly. An Endoglucanase, EglA, from the Hyperthermophilic Archaeon Pyrococcus furiosus Hydrolyzes 8-1,4 Bonds in Mixed-Linkage (1-3), (1-4)-R-D-Glucans and Cellulose. Journal of Bacteriology, 1990. Vol. 181 No.1 pg. 284-290.

K. Kretz, W. Callen, and V. Hedden. Cycle Sequencing. PCR Methods and Applications, 1994. Vol. 3 No. 5 Pg. S107.

W. Callen, V. Hedden, B. Jerpseth, J. Hayfield, J. Braman, and K. Kretz. The Clearcut Kit: A Versatile System for Rapid Plasmid Minipreps, Buffer Exchanges and PCR Cleanup. Strategies in Molecular Biology, 1993. Vol. 6 No. 2.V.

Hedden, W. Callen, J. M. Short, and K. Kretz. Improved Sequence Analysis of Mutations Identified with the Big Blue System. Strategies in Molecular Biology, 1993. Vol. 6 No.1.

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E. Marsh, W. Shoetllin, J. Cline, W. Callen, K. Kretz, and E. Mathur. Pyrococcus furiosus DNA Ligase and the Ligase Chain Reaction. Strategies in Molecular Biology, 1992. Vol. 5 No. 3.

K. Kretz, V. Hedden, and W. Callen. Cyclist DNA Sequencing Kit: Fast, Reliable Sequencing of Inconsistant Templates. Strategies in Molecular Biology, 1992. Vol. 5 No. 2.

E. J. Mathur, M. W. W. Adams, W. N. Callen, and J. M. Cline. The DNA Polymerase Gene from the Archaebacterium, Pyrococcus furiosis, Shows Sequence Homology with Alpha-Like DNA Polymerases. Nucleic Acids Research, 1991 Vol. 19 No. 24 Pg. 6952.

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J. Sorge, L. Blinderman, C. Katayama, W. Callen. ExoMeth Sequencing Method: Eliminates the Need for Subcloning and Oligonucleotide Primers. Strategies in Molecular Biology, 1990. Vol. 3 No. 1.

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E. Mathur, J. Cline, E. Marsh, W. Shoettlin, K. Nielson, B. Scott, W. Callen, K. Kretz, and J. Sorge. Isolation, Characterization and Cloning of DNA polymerase I and DNA ligase I from the marine hyperthermophile, Pyrococcus furiosis. American Society for Cell Biology Conference. Nov. '92.K.

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W. Callen, D. Shoemaker, E. Mathur, and J. Sorge. A Library of Synthetic Templates for the Directed Ligation of Any Two Oligonucleotide Hexamers. Cold Spring Harbor Human Genome Conference. April 1991.

D. Shoemaker, E. Mathur, W. Callen, J. Sorge. A Library of 256 Hexamers, Degenerate at Two Positions (5'-NNXXXX-3'), Can Create All Possible 12-mer Primers for Applications in High Volume DNA Sequencing Strategies. Cold Spring Harbor Human Genome Conference. April 1991.

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S.I. Bernstein, P.T. O'Donnell, K. Mogami, E.S. Roche, V. Collier, A. Fieck, W. Callen, W. Kronert, and E. McKennon. Molecular and Ultrastructural Analysis of Mutations in the Drosophila Muscle Myosin Heavy Chain Gene: Muscle-Specific Effects on Myosin Accumulation and Muscle Function. Gordon Research Conference on Molecular Genetics. August 1987.